

constructed conductance-voltage plots from macroscopic current or measured NPo from single channel recordings. Ethanol-induced potentiation of cbv1 currents turned into inhibition as Ca^{2+}_i increased, with a crossover at $\sim 20 \mu\text{M}$ Ca^{2+}_i . In contrast, slo2 and slo3 channels remained resistant to 50 mM ethanol even when probed at a wide concentration range of activating ion: 10-140 mM Na^{+}_i (slo2) and 0.01-10 μM OH^{-}_i (slo3). Data underscore that the ethanol-sensitivity and its ligand-dependence found in slo1 channels are not extensive to other members of the slo channel family, suggesting selective interaction(s) between Ca^{2+} -sensing and ethanol-sensing mechanisms. Support: R37AA011560 (AMD)

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Modulation of Hslo1 BK Channels by Aminoglycoside Antibiotics

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Large-conductance calcium- and voltage-activated potassium (BK) channels control smooth muscle tone and neurotransmitter release. BK channels consist of a voltage-sensing domain (VSD), a calcium-sensing domain (CSD), and a pore/gate domain (PGD). To elucidate structure and function of the so-called "C-linker" (res. #327 to 340 in human slo1 U11058) connecting PGD and CSD, we analyzed the impact of amino-acid exchanges within that area on inhibition of BK channels by the aminoglycoside antibiotic neomycin. Slo1 variants were expressed in HEK293 cells and analyzed in excised membrane patches. At a concentration of 100 μM , neomycin reversibly reduced hSlo1 BK currents at 200 mV to 30%. At low voltages, however, neomycin activated BK channels - a phenomenon largely masked by its profound pore blocking effect at high voltages. Assuming that the polycationic neomycin blocks the channel pore, we introduced mutations in the PGD increasing the net charge by one per subunit. For S317R, neomycin did not block at 200 mV and the voltage dependence of activation was left-shifted by almost 40 mV. For E324A, neomycin reduced the current at 200 mV to 81%, while a left-shift of 17 mV was measured. These data suggest that neomycin must have more than one interaction site with BK channels and that it not only blocks the pore but also interferes with gating. Moreover, alteration of the C-linker structure, which was known to be essential for channel gating, almost completely abolished BK inhibition by neomycin. The C-linker has thus to be considered a central part of the PGD, determining pore properties as well as channel gating.

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BK Channel Blockers Regulate Mouse Sinoatrial Node Cell Firing

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In the cardiovascular system, BK currents regulate vascular tone and may promote cardioprotection against ischemic injury. Recent experimental results also demonstrate that the BK channel blocker paxilline reduces the heart rate of mice in vivo, and in isolated rat hearts (Imlach, et al. 2010), suggesting a novel role for BK channels in basal heart rate regulation. However, the mechanism underlying this observation is unclear. We hypothesized that BK channel blockers reduce heart rate by altering electrical conduction within the heart. To test this hypothesis, we recorded electrocardiograms from awake, unrestrained mice in the presence of 8mg/kg paxilline, and examined changes in heart rate and cardiac conduction. Electrocardiograms show that the paxilline-induced reduction in heart rate in wild-type mice was associated with a $28.7 \pm 4.3\%$ ($n = 8$, $p = 0.0002$) elongation of the sinus (P-P) interval, suggesting that BK channels regulate cardiac pacing in vivo. Action potentials were recorded from isolated mouse sinoatrial node cells, and application of 3 μM paxilline reduced the average instantaneous firing frequency from $4.81 \pm 0.01\text{Hz}$ to $4.50 \pm 0.07\text{Hz}$ ($n = 8$, $p < 0.05$). The effect of PAX on firing frequency was alleviated by 1 μM isoproterenol (firing frequency = $5.28 \pm 0.02\text{Hz}$, $n=5$). Together, these results suggest that BK channels may influence basal cardiac function through a role in the sinoatrial node.

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Circadian Regulation of BK Channel Alternative Splicing in the Suprachiasmatic Nucleus

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The large-conductance calcium and voltage-activated potassium (BK) channel, encoded by *Kcnma1*, is alternatively spliced at 4 sites in the C-terminus. We investigated whether alternative splicing of *Kcnma1* in the suprachiasmatic nucleus (SCN), the brain's circadian clock, contributes to functional regulation of BK current. using real time semi-quantitative RT-PCR, we found increased expression of a 26-a.a. insert near the Ca^{2+} bowl (Site 3), and decreased expression of the C-terminal VEDEC sequence (Site 4) at night. To determine

the effect of these exons on BK currents, constructs containing inserts at Site 3 ($\alpha 26$ and $\alpha 0$) or Site 4 (α VEDEC and α VYR) were expressed in HEK293 cells. The voltage dependence of activation ($V_{1/2}$), and current kinetics (τ_{act} and τ_{deact}) were analyzed at multiple $[\text{Ca}^{2+}]_i$. Insertion of exon at Site 3 had no effect on current properties; τ_{act} , τ_{deact} , and $V_{1/2}$ were similar between $\alpha 26$ and $\alpha 0$ currents at each $[\text{Ca}^{2+}]_i$ ($n=8$ per condition). $V_{1/2}$ s for α VEDEC and α VYR currents were similar at 0, 1, and 10 μM ($n=8$ per condition), but the $V_{1/2}$ was left-shifted at 100 μM Ca^{2+} (α VEDEC= 12.5 ± 5.2 , $n=8$ versus α VYR= 47.8 ± 3.2 , $n=8$, $p=0.024$). While the VEDEC sequence had no effect on activation, τ_{deact} for α VEDEC currents were 30% faster at each $[\text{Ca}^{2+}]_i$. In addition, two BK variants were cloned from SCN differing at a 4-a.a. insert at Site 1 (α SRKR and α Insertless). Currents from α SRKR were left-shifted compared to α Insertless at 10 μM Ca^{2+} ($\Delta V_{1/2}=19.27$, $n=8$). However, when each variant was co-expressed with the $\beta 4$ subunit, $\Delta V_{1/2}$ increased to 50.9 ($n=5$), suggesting beta subunits may contribute to the expression of BK splice variant differences. These findings suggest that the SCN exhibits circadian regulation of *Kcnma1* alternative splicing and that splicing contributes to differences in BK current properties.

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Structural Studies of the Apo and Ca^{2+} -Bound States of the Human BK (SLO1) Channel Gating Ring in Solution

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The gating ring (GR) regulates the activity of large-conductance voltage- and Ca^{2+} -activated K^{+} channels (BK) by interacting with intracellular signaling molecules. To understand the operation of this biological sensor under physiological conditions, we performed Small-Angle X-ray Scattering (SAXS) analysis, at beamline 4-2 at the Stanford Synchrotron Radiation laboratory. SAXS measurements of the purified GR were performed in the absence or in the presence of 35 μM free Ca^{2+} , found to be a saturating concentration in previous work. The quality of the circularly-averaged scattering data was evaluated with Guinier analysis, while the ATSAS software suite was used to derive structural information. The radius of gyration (R_g) and maximum interparticle distance (D_{max}) of the apo GR were $48.65 \pm 1.372 \text{ \AA}$ and 185 \AA , respectively. These values are comparable to data obtained from crystal structure of GR (3NAF), where the envelope R_g , calculated with CRY SOL, is 45.55 \AA , and its diameter 155.6 \AA . Ca^{2+} -bound GR shows a decrease in R_g to $42.77 \pm 1.058 \text{ \AA}$ and D_{max} to 160 \AA , demonstrating the structural response of GR to Ca^{2+} . Low-resolution structural models of the GR were generated from the experimental scattering pattern using DAMMIN. The Ca^{2+} -bound GR revealed notable changes in both flexible and assembly interfaces of the superstructure's constituent RCK1 (Regulator of Conductance for K^{+}) and RCK2 domains. Since the structural changes are resolved under physiologically-relevant conditions, we speculate that they represent the molecular transitions that initiate the Ca^{2+} -induced activation of human BK channels.

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Single Aspartate Substitutions at Multiple Deep-Pore Positions Lead to Constitutively Open BK Channels

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The pore-lining amino acids of ion channel proteins reside on the interface between a polar (the pore) and a non-polar environment (the rest of the protein). Manipulating the charge states of their side-chains, by tilting the energy balance between a buried and a pore-exposed conformation, can reveal important details about gating-related structural dynamics. For example, charged side-chains at the M314 position in the BK channel stabilize an open conformation. The M314D mutant, in particular, led to constitutive channel activities. We have now scanned the S6 region (I308 to N328) with aspartate replacement. We found 3 other positions where aspartate substitutions led to constitutively open BK channels in nominal zero calcium and neutral pH. These mutants are L312D, A313D and A316D. Together with M314, these positions are all located within the deep-pore region of the channel, which is immediately intracellular to the selectivity filter but more extracellular to the lower-pore region that aligns with the Kv channel "bundling crossing". These findings provide further evidence that the BK channel pore is different from that of Kv channels, where aspartate substitution at a single position (as opposed to multiple positions in BK) in the lower-pore region (as opposed to the deep-pore region in BK) led to constitutive channel activities. The aspartate mediated constitutive activities in Kv channels is believed to result from the disruption of specific hydrophobic